

The University of Chicago

STUDIES ON THE TRANSMISSION OF  
LYMPHOCYTIC CHORIOMENINGITIS  
VIRUS BY ARTHROPODS

A PART OF A DISSERTATION SUBMITTED TO  
THE FACULTY OF THE DIVISION OF THE  
BIOLOGICAL SCIENCES IN CANDIDACY FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1941

By  
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# STUDIES ON THE TRANSMISSION OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS BY ARTHROPODS

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The natural mode of transmission of the virus of lymphocytic choriomeningitis either in man or animals is unknown. The present studies were undertaken to study the behavior of this virus in certain arthropods, to discover, if possible, its natural vectors. The arthropods tested were *Aedes aegypti*, *Culex pipiens*, *Aedes albopictus*, bedbugs (*Cimex lectularius*), rhesus monkey lice (*Eupedicinus longiceps*) and a blood-sucking mite (*Atricholaelaps glasgowi*).

Although the virus is excreted in the urine<sup>1</sup> and nasal secretions<sup>1,2</sup> of susceptible laboratory animals and man, various workers<sup>3,4,5,6</sup> have reported that the infection is not readily transmitted by contact or feeding. Traub<sup>7</sup> and Haas,<sup>8</sup> however, have shown that white mice convey infection to their offspring in utero and in infancy and that naturally infected mice transmit infection by contact more readily than do artificially infected animals. Evidence that gray mice (*Mus musculus*) are an effective reservoir of the choriomeningitis virus from which man may become

infected is shown by the recent work of Armstrong, Wallace, and Ross.<sup>9</sup> These workers recovered the virus from mice trapped in the homes of 4 proven cases of lymphocytic choriomeningitis in Washington, D. C., and of 1 in Lancaster, Pa., while there were no cases among homes which harbored noninfected mice or a larger proportion of homes harboring no mice. Furthermore, Wooley and his associates<sup>10</sup> noted a relatively higher incidence of virus neutralizing antibodies in those groups of people which have a greater opportunity for contact with infected gray mice or laboratory animals. There is also evidence that dogs might serve as a reservoir of the virus.<sup>11</sup> Besides man, gray mice, and dogs, albino mice<sup>12,13</sup> and rhesus monkeys (*Macaca mulatta*)<sup>14</sup> have been found spontaneously infected with lymphocytic choriomeningitis.

The possibility of a venereal route of transmission has been suggested by Wooley, Armstrong and Onstott.<sup>10</sup> These workers were able to transmit infection occasionally by instilling the virus into the urethra or vagina of monkeys. They also demonstrated the presence of the virus in the seminal fluid and testicular tissue of infected animals. The respiratory tract has also been suggested as a possible route of infection because of the influenza-like symptoms frequently associated with the disease in man and

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1. Traub, E.: J. Exper. Med. **63**: 533, 1936.
2. MacCallum, F. O., and Findlay, G. M.: Lancet **1**: 1370, 1939.
3. Rivers, T. M., and Scott, T. F. McN.: J. Exper. Med. **63**: 415, 1936.
4. Traub, E.: J. Exper. Med. **64**: 183, 1936.
5. Shaughnessy, H. J., and Milzer, A.: Am. J. Pub. Health **29**: 1103, 1939.
6. Shaughnessy, H. J., and Zichis, J.: J. Exper. Med. **72**: 331, 1940.
7. J. Exper. Med. **69**: 801, 1939.
8. Pub. Health Rep. **56**: 285, 1941.

9. Pub. Health Rep. **55**: 1222, 1940.
10. Pub. Health Rep. **52**: 1105, 1937.
11. Dalldorf, G.: J. Exper. Med. **70**: 19, 1939.
12. Traub, E.: Science **81**: 298, 1935; Lépine, P., and Sautter, V.: Compt. rend. Acad. d. sc. **202**: 1624, 1936.
13. Findlay, G. M., Alcock, N. S., and Stern, R. O.: Lancet **1**: 650, 1936.
14. Armstrong, C., and Wooley, J. G.: Pub. Health Rep. **50**: 537, 1935.



the fact that the virus has been isolated from the nasopharynx.<sup>2</sup> In addition, mice have been infected by nasal instillation of the virus.<sup>13</sup> A review of the literature thus far, however, indicates that the disease is sporadic in man and that secondary cases do not occur. On the other hand, the possibility of an arthropod vector is suggested by many of the above findings and the constant presence of the virus in high concentration in the blood stream of infected animals<sup>3,5,15,16</sup> and man.<sup>17</sup> Moreover, infection is readily transmitted by the subcutaneous route of inoculation<sup>1,3</sup> or when the virus is placed on the lightly scarified skin of guinea pigs,<sup>5</sup> white mice, and rhesus monkeys<sup>13</sup> or on the normal, unscarified skin of guinea pigs.<sup>6</sup>

In 1939 Coggeshall<sup>16</sup> announced the successful transmission of lymphocytic choriomeningitis from guinea pig to guinea pig through the bites of *Aedes aegypti*. These mosquitoes were capable of transmitting the virus as early as the fourth day and as late as the fifteenth day after feeding on an infected animal. In the same year Shaughnessy and Milzer<sup>5</sup> reported experimental infection of all stages of the life cycle of the Rocky Mountain wood tick (*Dermacentor andersoni* Stiles). They demonstrated transmission of the virus from larvae to nymphs, nymphs to adults, and transmission of the virus from adults to eggs and subsequent larvae. They were unable to transmit the infection by feeding adult ticks which had been infected either in the nymphal or adult stages. Infection was transmitted, however, by feeding nymphs which had engorged in their larval stage on an infected guinea pig. Infection was also transmitted by applying infected crushed ticks or feces

from ticks which had engorged in their previous stage upon infected animals to the scarified skin of normal guinea pigs.

#### METHODS AND MATERIALS

*Laboratory animals.*—Guinea pigs ranging in weight from 300 to 500 gm. were used in the majority of the transmission experiments. Daily temperature and weight records as well as observations were made. The possibility of intrarectal transmission of the virus reported by Shaughnessy and Zichis<sup>6</sup> was eliminated by the use of rectal thermometers disinfected in 10% formalin and rinsed in tap water before using. The temperatures of normal animals and animals used in arthropod transmission experiments were taken with individual thermometers. At necropsy, portions of the brain and lungs of each animal were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin for histological study. In all positive transmission experiments, however, identification of recovered virus was established by means of a specific serum neutralization test because pathological findings are not sufficiently characteristic to differentiate some of the virus diseases that attack the central nervous system of laboratory animals.

Albino Swiss mice, between 4 and 5 weeks of age, were used for studies of the virus in the blood stream, and also in some of the arthropod transmission experiments. Three rhesus monkeys (*Macaca mulatta*), weighing 3.21 to 4.10 kilograms, were used in transmission experiments with monkey lice. These animals had been previously inoculated with poliomyelitis virus but recovered and were readily susceptible to lymphocytic choriomeningitis when inoculated subcutaneously with known virus.

In order to prove that the strains of stock guinea pigs and mice used in subsequent work were free from latent in-

15. Traub, E.: J. Exper. Med. **63**: 847, 1936.

16. Coggeshall, L. T.: Science **89**: 515, 1939.

17. Lépine, P., and Sautter, V.: Ann. Inst. Pasteur **61**: 519, 1938.



fection with lymphocytic choriomeningitis virus, 12 guinea pigs and an equal number of mice were inoculated intracerebrally with 2% corn starch (Kingsford) suspended in 0.85% salt solution—a procedure which has been shown by Findlay, Alcock, and Stern<sup>13</sup> to activate a subclinical infection of this virus into a typical syndrome. All animals inoculated with starch remained normal during the 30 days they were kept under observation. On the other hand, no stock animals were found that were immune to inoculation with a fatal dose of virus. Furthermore, in the course of another study where 52 specimens of cerebrospinal fluid from various central nervous system infections in man were inoculated subcutaneously into guinea pigs and intracerebrally into mice, none of the animals developed lymphocytic choriomeningitis infection or immunity.<sup>18</sup> No virus neutralizing antibodies were found in the blood of rhesus monkeys used in monkey lice transmission experiments prior to inoculation with the virus.

The above evidence seemed sufficient proof that the stock animals were not naturally infected with the virus. No ectoparasites were noted on stock mice and guinea pigs, and as a further precaution new shipments of stock animals were dipped in 2% cresol and washed in water when received. All test animals used in arthropod transmission experiments were kept in isolated individual metal cages which were disinfected 3 times weekly with 5% cresol solution. The animals were housed in new quarters free from infestation with bedbugs, cockroaches, house mice, and domestic rats so that the possibility of animals becoming infected from these sources was minimized.

*Virus strain.*—The J.P. strain of lymphocytic choriomeningitis virus used in these studies was isolated in January, 1939, from the cerebrospinal fluid of a patient suffering from recurrent lymphocytic choriomeningitis. The immunological properties of this strain have been described in a previous paper.<sup>18</sup> When the present studies were begun the virus had undergone 40 guinea pig passages by subcutaneous inoculation.

Subcutaneous inoculation of guinea pigs with 0.5 cc. of a 10% infected guinea pig brain suspension usually resulted in rise in temperature (104–105.5 F.) within 48 to 72 hours and death in 8 to 10 days. The temperature generally remained high until about 24 hours before death when it dropped below normal. The guinea pigs lost weight rapidly, showed labored respiration, and generally developed terminal salivation. Frequently they lost as much as a third of their body weight before death. The course of the disease was similar in guinea pigs infected in the positive arthropod transmission experiments except that the incubation period and length of life was prolonged and more variable. Pyrexia occurred 7 to 18 days after exposure, and death ensued in 16 to 27 days.

At necropsy the brain appeared grossly hyperemic and areas of discoloration and consolidation were seen in the lungs. Microscopically, there was slight infiltration of the meninges with lymphocytes and a small percentage of plasma cells and polymorphonuclear leukocytes (fig. 1). Stained sections of the consolidated areas in the lungs showed an interstitial bronchopneumonia (fig. 2) which, according to Rivers and Scott,<sup>3</sup> is similar to that caused by many other viruses. No definite pathological changes were noted in other organs, except a few areas of focal necrosis in the liver. These postmortem

18. Leichenger, H., Milzer, A., and Lack, H.: J.A.M.A. 115: 436, 1940.



findings agree with those of Rivers and Scott<sup>2</sup> and Findlay and Stern.<sup>19</sup>

Typical signs of lymphocytic choriomeningitis in mice inoculated intracerebrally occur after an incubation period of 6 to 7 days and consist of a ruffled coat, inactivity, and loss of appetite.<sup>3</sup> Later, the mice show marked tremors of the limbs especially when lifted by the tail. Death usually occurs 8 to 10 days after intracerebral inoculation and is often accompanied by convulsions with characteristic opisthotonus and rigid extension of the hind legs. The same type of pathological lesions were seen in mice inoculated intracerebrally as noted for the guinea pig but lymphocytic infiltration of the meninges and choroid plexus and thickening of the meninges were more marked.

*Identification of the virus.*—Virus recovered in successful arthropod transmission experiments was shown to be lymphocytic choriomeningitis by means of a neutralization test with a specific human convalescent serum. This serum was obtained from the writer who was accidentally infected in 1939. The virus of lymphocytic choriomeningitis was isolated from the writer's cerebrospinal fluid, and there subsequently developed a strong concentration of both complement-fixing and neutralizing antibodies against the isolated and J.P. strains of virus followed by uneventful recovery.<sup>20</sup>

The neutralization test employed was a slight modification of the technic described by Baird and Rivers.<sup>21</sup> Equal parts of  $10^{-2}$  and  $10^{-3}$  saline dilutions of infected guinea pig brain suspensions and undiluted immune serum were mixed thoroughly and incubated at 37 C. for 1 hour. Simultaneously control tubes containing virus plus serum of a

child under 5 or buffered salt solution were prepared.\* Positive controls containing immune serum plus known virus were included in selected tests. Four guinea pigs were used in each neutralization test. Five-tenths cc. of each serum-

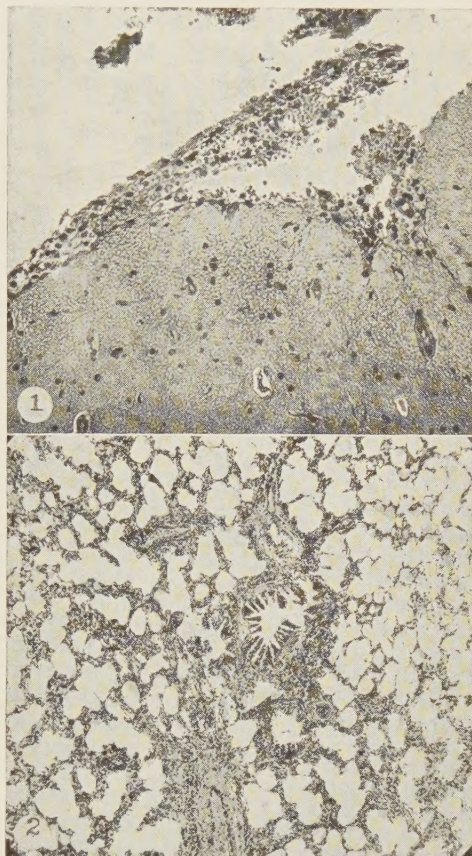


Fig. 1.—Section of the brain of a guinea pig bitten 14 days previously by infected *A. aegypti* showing meningoencephalitis with lymphocytic infiltration, engorgement of meningeal blood vessels, and perivascular infiltration. Hematoxylin and eosin.  $\times 168$ .

Fig. 2. Section of lung of same guinea pig showing interstitial bronchopneumonia. Hematoxylin and eosin.  $\times 56$ .

19. J. Path. & Bact. **43**: 327, 1936.

20. Milzer, A., and Levinson, S. O.: Unpublished studies.

21. Am. J. Pub. Health **28**: 47, 1938.

\* No serum of a child under 5 years was found that would neutralize the virus. The serums were obtained from children patients in Michael Reese Hospital. In other tests it was found that serum of normal monkeys or guinea pigs would not inactivate the virus.



virus or salt solution-virus mixture was injected subcutaneously into guinea pigs, and the animals were observed for 30 days. In positive tests animals inoculated with virus plus normal serum or salt solution succumbed with typical signs of lymphocytic choriomeningitis in 8 to 12 days,\* while animals inoculated with virus plus immune serum remained normal. During the early stages of the present studies the immune serum was of such potency that it would neutralize a  $10^{-1}$  saline dilution of infected guinea pig brain suspension. Later it was necessary to use  $10^{-3}$  and  $10^{-4}$  instead of  $10^{-2}$  and  $10^{-3}$  virus dilutions in the neutralization tests because titration experiments indicated a decrease in titer of virus neutralizing substances present in the writer's immune serum.

After being observed for 30 days, surviving guinea pigs were tested for lymphocytic choriomeningitis immunity by subcutaneous inoculation with a challenge dose consisting of 0.5 cc. of a 10% guinea pig brain suspension infected with the J. P. strain of virus.

*Mosquitoes.*—The mosquitoes used in these studies were bred, hatched, and caged according to Huff's technic.<sup>22</sup> *A. aegypti* eggs were supplied by Dr. William Trager; the *Culex pipiens* eggs were obtained from Prof. Clay G. Huff; and *Aedes albopictus* eggs were sent by the Army Medical School, Washington, D. C. Between blood meals these insects were fed raisins which had been boiled several minutes in sugar solution.

\* It was noted, however, that control guinea pigs inoculated with high dilutions of virus ( $10^{-4}$  and  $10^{-5}$ ) sometimes had prolonged and variable incubation periods and length of life in contrast to guinea pigs inoculated with lower dilutions of virus.

22. In Galtsoff, P. S., Lutz, F. E., Welch, P. S., and Needham, J. G.: *Culture Methods for Invertebrate Animals*, Ithaca, N. Y., Comstock Publishing Co., 1937, p. 386.

For transmission experiments unfed females were allowed to engorge on a guinea pig inoculated subcutaneously 7 to 11 days previously with 0.5 cc. of  $10^{-1}$  saline dilution of virus infected guinea pig brain suspension. Before exposure to the mosquitoes, the guinea pig was immobilized on a small animal board, and its belly was shaved. The lantern globe cage containing the mosquitoes was then inverted and suspended from an iron ring attached to a ring stand so that the netting-covered mouth of the globe was about 1 or 2 mm. above the guinea pig's belly, in order that the mosquitoes could feed by inserting their proboscides through the netting. As a rule nearly all of the mosquitoes engorged in 30 to 45 minutes; however, *A. albopictus* and *C. pipiens* did not feed as readily as *A. aegypti*. Moreover, it was necessary to feed *C. pipiens* in a dark room. The latter species was exposed continuously to electric light between feedings. All females which failed to feed were discarded. To prevent infection from the feeding apparatus, the mouth of the lantern globe was disinfected with 5% cresol solution, washed with water, and dried soon after each feeding. A clean piece of gauze was also placed over the netting cover of the globe.

Mosquitoes were allowed to bite normal animals at varying intervals after the infecting meal in order to test their ability to transmit the virus under consideration. The fed insects were also tested for the presence of the virus after the same time intervals by grinding them in a mortar with physiological salt solution, filtered through several layers of gauze, and injected subcutaneously into guinea pigs. The mosquito species tested were kept at room temperature (22–25 C.); later the effect of temperature on transmission of the virus by *A. aegypti* was tested by incubating, immediately after feeding on infected



guinea pigs for varying periods of time, inconstant-temperature incubators with an accuracy of at least  $\pm 0.25$  C.<sup>23</sup> The following temperatures were used—25, 26, 27, 28, 30, 32, 34, and 37 C. No attempt was made to measure the humidity in the lantern globe cages containing mosquitoes, but the relative humidity in the incubators ranged from 59 to 62%.

*Bedbugs.*—Except for a few preliminary experiments most of the bedbugs (*Cimex lectularius*) used were laboratory bred descendents of adult bedbugs collected from animal cages of another laboratory. The bugs were stored and reared in cotton-plugged sterile test tubes at room temperature (22–25 C.). A strip of sterile filter paper was placed in each test tube on which the bugs could rest and lay eggs. Feeding was accomplished by holding the mouth of the test tube containing bugs against the shaven belly of an immobilized guinea pig. Stock guinea pigs upon which non-infected bedbugs fed or were inoculated as ground saline suspensions remained healthy. Later they succumbed with typical signs of choriomeningitis when inoculated with the virus.

Bedbugs, like mosquitoes, were applied to the belly of an infected guinea pig 5 to 9 days after inoculation and were subsequently tested for infectivity at various intervals by being fed on normal animals or inoculated subcutaneously with ground saline suspensions or both. Similar precautions were taken to prevent spread of infection by the feeding apparatus as already described in the technic for feeding mosquitoes. Furthermore, the bugs were transferred to a new sterile test tube after each infecting meal. Attempts to transmit the virus by the oral route were made by forcing guinea pigs to swallow no. 5 gelatin cap-

sules containing living, infected bedbugs.

*Monkey lice.*—The monkey lice (*Eupedicinus longiceps* (Paiget)) were obtained from monkeys convalescent from poliomyelitis that did not possess demonstrable lymphocytic choriomeningitis virus-neutralizing antibodies in their blood serum. The lice and mites used in this and the following experiment were identified through the kindness of Dr. H. E. Ewing of the United States Bureau of Entomology and Plant Quarantine. After feeding for several days upon monkeys inoculated subcutaneously with the virus, the lice were tested for infectivity by uncontrolled feeding on a normal monkey and subcutaneous inoculations of ground saline suspensions into normal guinea pigs.

*Mites.*—The species of blood-sucking mite (*Atricholaelaps glasgowi* (Ewing)) tested were obtained from a field mouse nest sent through the courtesy of Dr. Irving Fox of Iowa State College. These mites were successfully bred and stored at room temperature (22–25 C.) in a glass battery jar containing a normal mouse upon which they could feed. The inner surface of the jar near the top was ringed with a thick layer of vaseline to prevent mites from escaping; the bottom of the jar was covered with approximately 2 inches of sawdust mixed with remains of the original mouse nest to absorb mouse excreta and furnish a breeding place for the mites. A tightly woven cloth covered the opening of the jar, and food for the mouse was introduced through a slit cut in the cloth and sealed with adhesive tape. After the mites had increased in sufficient numbers, some of them were transferred to a second similarly prepared battery jar containing an infected mouse. After the mouse died the mites were collected from hairs with a pair of sterile forceps and were tested for infectivity by being

23. Huff, C. G: Am. J. Hyg. 32: 71. 1940.



injected as a ground saline suspension or transferred at various intervals to the hair of other healthy mice. Control mice, housed in jars containing excreta of recently removed infected mice developed neither lymphocytic choriomeningitis infection nor immunity. Transmission experiments were carried out with mites kept at room temperature. Gelatin capsules containing living, infected mites were also fed to normal guinea pigs and mice.

Ewing<sup>24</sup> has stated that this species of mite is the most common ectoparasite of small North American mammals. He further stated that there are records of it from all parts of the United States, though little is known of its habits.

*Cultural studies.*—Virus suspensions obtained from the brain of experimental animals were cultured aerobically and anaerobically on blood agar plates and by the inoculation of Rosenow's brain medium. These mediums were then incubated at 37 C. for 7 days. Only 2 of 60 virus-infected guinea pig brain suspensions were contaminated, and nonpathogenic *Staphylococcus albus* was isolated in each instance.

Selected cultures were made of ground arthropod saline suspensions and bed-bug feces in Rosenow's brain medium incubated at 37 C. and Smith tubes containing nutrient broth incubated at room temperature for 7 days.

#### EXPERIMENTAL RESULTS

##### Persistence of the Virus in the Blood Stream of an Infected Guinea Pig and Albino Swiss Mice

In order to find the optimum time for feeding arthropods on infected guinea pigs and mice, experiments were carried out to determine the initial appearance and the persistence of the J. P. strain of lymphocytic choriomeningitis virus in the blood stream following various

routes of inoculation. Rivers and Scott<sup>8</sup> stated that the blood of guinea pigs inoculated intracerebrally was infectious for mice at least as early as the fourth day after inoculation. Traub<sup>15</sup> showed that the virus persisted in the blood stream of mice for weeks or months after recovery, and Findlay, Alcock, and Stern<sup>13</sup> found the virus present at death in the blood of intracerebrally inoculated mice. Recently Shaughnessy and Milzer<sup>5</sup> showed that active virus appeared in the blood of guinea pigs 24 hours after combined intracerebral and subcutaneous inoculation of the W. E. (Rivers) strain and persisted until the death of the animals. No studies have been reported in the literature, however, concerning the initial appearance of the virus in the blood of guinea pigs following subcutaneous inoculation, or in infected mice following any route of inoculation.

One experiment was done showing the initial appearance and persistence of the J. P. strain in the blood stream of a guinea pig inoculated subcutaneously with 0.5 cc. of a 10% infected guinea pig brain suspension (table 1). The animal was bled daily by cardiac puncture. The concentration of virus in the blood stream was determined by inoculating intracerebrally 0.03 cc. of heart blood in 1% solution of sodium citrate diluted with buffered salt solution into 2 mice for each dilution tested. Results in table 1 show that the virus was detected in the blood stream of this guinea pig 24 hours after inoculation.\* The virus concentration in the blood stream gradually increased, reaching a peak about 5 days after inoculation and persisted until the death of the animal.

\* Since these experiments were completed, Dr. H. J. Shaughnessy of the Illinois Department of Public Health has stated that he obtained similar results in unpublished experiments with the W. E. (Rivers) virus strain.

24. Personal communication.



The next 3 experiments were carried out to determine the initial appearance and persistence of the virus in the blood stream of albino Swiss mice inoculated by the intracerebral, subcutaneous, and the intraperitoneal routes respectively. In each experiment daily blood samples drawn from the tails of 3 mice were pooled in Wassermann tubes containing 2% potassium oxalate, and 0.15 cc. of each pool was injected subcutaneously into a normal guinea pig. Results tabulated in table 2 show that blood obtained from mice inoculated intracerebrally (0.03 cc. of 10% infected guinea pig brain suspension) was infectious 24 hours after inoculation but was present in such small amounts that 21 days were required to kill a guinea pig. Data also in table 2 show that the virus appeared

TABLE 1.—*Persistence of the Virus of Lymphocytic Choriomeningitis in the Blood Stream of Infected Guinea Pig<sup>a</sup> Following Subcutaneous Inoculation*

| Day Following Inoculation | Virus Dil.       | Day of Death of Mice |
|---------------------------|------------------|----------------------|
| 1                         | 10 <sup>-1</sup> | 0, + <sup>b</sup>    |
|                           | 10 <sup>-2</sup> | 0, 0                 |
|                           | 10 <sup>-3</sup> | 0, 0                 |
| 2                         | 10 <sup>-1</sup> | 8, +                 |
|                           | 10 <sup>-2</sup> | 0, 0                 |
|                           | 10 <sup>-3</sup> | 0, 0                 |
| 3                         | 10 <sup>-1</sup> | 8, 8                 |
|                           | 10 <sup>-2</sup> | 14, +                |
|                           | 10 <sup>-3</sup> | 0, 0                 |
| 4                         | 10 <sup>-1</sup> | 9, 14                |
|                           | 10 <sup>-2</sup> | 15, 16               |
|                           | 10 <sup>-3</sup> | +, +                 |
| 5                         | 10 <sup>-1</sup> | 9, 10                |
|                           | 10 <sup>-2</sup> | 9, 9                 |
|                           | 10 <sup>-3</sup> | 9, +                 |
| 6                         | 10 <sup>-1</sup> | 7, 8                 |
|                           | 10 <sup>-2</sup> | 11, +                |
|                           | 10 <sup>-3</sup> | 10, 11               |
| 7                         | 10 <sup>-1</sup> | 8, 9                 |
|                           | 10 <sup>-2</sup> | 9, 10                |
|                           | 10 <sup>-3</sup> | +, +                 |
| 8                         | 10 <sup>-1</sup> | 7, 9                 |
|                           | 10 <sup>-2</sup> | 8, 8                 |
|                           | 10 <sup>-3</sup> | 7, 7                 |
| Control <sup>c</sup>      | 10 <sup>-2</sup> | 0, 0                 |

<sup>a</sup> This animal was found dead on the ninth day following virus inoculation.

<sup>b</sup> Indicates number of surviving mice that developed typical signs of choriomeningitis but recovered.

<sup>c</sup> The control mice were inoculated with heart blood drawn just before injecting the guinea pig with virus. Later the control mice succumbed to inoculation with a challenge dose of virus.

TABLE 2.—*Persistence of the Virus of Lymphocytic Choriomeningitis in the Blood Stream of Albino Swiss Mice Inoculated by Various Routes*

| Guinea Pig No.                                                | Day Following Inoculation | Days Before Death |
|---------------------------------------------------------------|---------------------------|-------------------|
| Inocula from 3 Mice Inoculated Intracerebrally <sup>a</sup>   |                           |                   |
| 73                                                            | 1                         | 21                |
| 78                                                            | 2                         | 11                |
| 64                                                            | 3                         | 12                |
| 85                                                            | 4                         | 14                |
| 105                                                           | 5                         | 12                |
| 89                                                            | 6                         | 10                |
| 77                                                            | 7                         | 11                |
| 102                                                           | Control <sup>b</sup>      | 0                 |
| Inocula from 3 Mice Inoculated Subcutaneously <sup>a</sup>    |                           |                   |
| 122                                                           | 1                         | 9                 |
| 114                                                           | 2                         | 9                 |
| 124                                                           | 3                         | 9                 |
| 99                                                            | 4                         | 8                 |
| 125                                                           | 5                         | 11                |
| 126                                                           | 6                         | 10                |
| 127                                                           | 7                         | 11                |
| 132                                                           | 8                         | 9                 |
| 101                                                           | 14                        | 10                |
| 98                                                            | Control <sup>b</sup>      | 0                 |
| Inocula from 3 Mice Inoculated Intraperitoneally <sup>a</sup> |                           |                   |
| 109                                                           | 1                         | 11                |
| 110                                                           | 2                         | 11                |
| 112                                                           | 3                         | 10                |
| 113                                                           | 4                         | 8                 |
| 114                                                           | 5                         | 9                 |
| 115                                                           | 6                         | 9                 |
| 116                                                           | 7                         | 8                 |
| 87                                                            | Control <sup>b</sup>      | 0                 |

<sup>a</sup> Intracerebrally inoculated mice developed typical signs of choriomeningitis and 2 died in 7 and 8 days; the third recovered. Only 1 mouse in each of 3 inoculated by the subcutaneous and intraperitoneal routes respectively succumbed (9 and 12 days) with typical signs of choriomeningitis. The 4 remaining mice showed only rough coats and recovered.

<sup>b</sup> Control guinea pigs were inoculated with pooled blood drawn just before injecting the mice with virus. Later the control guinea pigs succumbed to inoculation with a challenge dose of virus.

in the blood stream of infected mice in high concentration 24 hours after inoculating subcutaneously or intraperitoneally 0.5 cc. of a 10% infected guinea pig brain suspension. It is interesting to note that after clinical recovery virus was still present in the blood of a mouse inoculated subcutaneously 14 days previously.

#### Rôle of Mosquito Incubation Temperature in Transmission of the Virus by *A. aegypti*

Four attempts to confirm Coggeshall's report<sup>16</sup> that he was able to transmit lymphocytic choriomeningitis from guinea pig to guinea pig by the bite of



*A. aegypti* (Trager strain) gave uniformly negative results. Since the importance of incubation temperature was not fully appreciated, the mosquitoes were kept at room temperature (22–25 C.) in the preliminary experiments. Four lots with 9 to 12 mosquitoes in

cross-immunity tests to be immunologically identical. For this reason and the fact that the Coggeshall strain produced both a clinical and pathological picture in guinea pigs and Swiss mice similar to that of the J. P. strain, it was considered unnecessary to repeat insect

TABLE 3.—Effect of Mosquito Incubation Temperature on the Transmission of Lymphocytic Choriomeningitis by *Aedes aegypti*

| Mosquito Lot No. | Days between Inoculation and Feeding on Infected Guinea Pig <sup>a</sup> | Mosquito Incubation Temperature C. | Days Since Infective Blood Meal | No. Feeding on Normal Guinea Pig | Result of Feeding <sup>b</sup> |                 | No. Mosquitoes Inoculated <sup>c</sup> | Result of Mosquito Inoculation <sup>b</sup> |                 |
|------------------|--------------------------------------------------------------------------|------------------------------------|---------------------------------|----------------------------------|--------------------------------|-----------------|----------------------------------------|---------------------------------------------|-----------------|
|                  |                                                                          |                                    |                                 |                                  | Days before Death              | Virus Recovered |                                        | Days before Death                           | Virus Recovered |
| 1                | 8                                                                        | 22–25                              | 5                               | 11                               | Lived                          |                 | 17                                     | Lived                                       |                 |
| 2                | 9                                                                        | 22–25                              | 9                               | 9                                | Lived                          |                 | 25                                     | Lived                                       |                 |
| 5                | 10                                                                       | 22–25                              | 14                              | 12                               | Lived                          |                 | 19                                     | Lived                                       |                 |
|                  | (1st feeding 3/4)                                                        |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
|                  | 8                                                                        |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
|                  | (2nd feeding 3/8)                                                        |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
|                  | 4                                                                        |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
| 6                | (3rd feeding 3/11)                                                       | 22–25                              | 21                              | 11                               | Lived                          |                 | 14                                     | Lived                                       |                 |
|                  | 8                                                                        |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
|                  | (1st feeding 3/8)                                                        |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
|                  | 5                                                                        |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
|                  | (2nd feeding 3/13)                                                       |                                    |                                 |                                  |                                |                 |                                        |                                             |                 |
| 10               | 9                                                                        | 25                                 | 7                               | 13                               | Lived                          |                 | 13                                     | Lived                                       |                 |
| 11               | 9                                                                        | 25                                 | 14                              | 13                               | 13                             | 0               | 15                                     | Lived                                       |                 |
| 12               | 9                                                                        | 25                                 | 21                              | 14                               | Lived                          |                 | 17                                     | Lived                                       |                 |
| 16               | 7                                                                        | 26                                 | 7                               | 8                                | 21                             | +               | 13                                     | Lived                                       | Immune          |
| 17               | 7                                                                        | 26                                 | 14                              | 18                               | 25                             | +               | 21                                     | 14                                          | +               |
| 18               | 7                                                                        | 26                                 | 21                              | 12                               | Lived                          |                 | 13                                     | 30                                          | +               |
| 13               | 7                                                                        | 27                                 | 7                               | 9                                | Lived                          |                 | 11                                     | Lived                                       |                 |
| 14               | 7                                                                        | 27                                 | 14                              | 10                               | Lived                          |                 | 14                                     | Lived                                       |                 |
| 15               | 7                                                                        | 27                                 | 21                              | 12                               | Lived                          |                 | 16                                     | Lived                                       |                 |
| 7                | 7                                                                        | 28                                 | 7                               | 14                               | 27                             | +               | 14                                     | 25                                          | +               |
| 8                | 7                                                                        | 28                                 | 14                              | 12                               | 17                             | +               | 13                                     | 20                                          | +               |
| 9                | 7                                                                        | 28                                 | 21                              | 18                               | 17                             | +               | 23                                     | 20                                          | +               |
| 19               | 6                                                                        | 30                                 | 7                               | 1                                | 18                             | +               | 7                                      | 27                                          | +               |
| 20               | 6                                                                        | 30                                 | 14                              | 4                                | 21                             | +               | 9                                      | Lived                                       | Immune          |
| 21               | 6                                                                        | 30                                 | 21                              | 5                                | 18                             | +               | 9                                      | 29                                          | +               |
| 28               | 8                                                                        | 30                                 | 38                              | 5                                | 18                             | +               | 5                                      | Lived                                       | +               |
| 22               | 9                                                                        | 32                                 | 7                               | 6                                | Lived                          |                 | 12                                     | Lived                                       |                 |
| 23               | 9                                                                        | 32                                 | 14                              | 4                                | 18                             | +               | 9                                      | 19                                          | +               |
| 24               | 7                                                                        | 32                                 | 21                              | 8                                | 18                             | +               | 11                                     | 17                                          | +               |
| 25               | 8                                                                        | 34                                 | 8                               | 11                               | 19                             | +               | 15                                     | Lived                                       | Immune          |
| 26               | 8                                                                        | 34                                 | 15                              | 7                                | Lived                          |                 | 10                                     | Lived                                       |                 |
| 27               | 8                                                                        | 34                                 | 22                              | 10                               | Lived                          |                 | 12                                     | Lived                                       |                 |
| 19A              | 9                                                                        | 37                                 | 7                               | 9                                | Lived                          |                 | 14                                     | Lived                                       |                 |

<sup>a</sup> Infected guinea pigs were inoculated subcutaneously (0.5 cc.) with 10 % virus suspension.  
<sup>b</sup> In negative experiments guinea pigs subsequently succumbed with typical signs of choriomeningitis following inoculation with a challenge dose of virus.  
<sup>c</sup> Mosquitoes that fed together with those that failed to feed were ground in salt solution immediately after each feeding experiment and injected subcutaneously into a guinea pig.

each were allowed to bite an equal number of normal guinea pigs at intervals ranging from 5 to 21 days after the infecting meal (table 3). In order to increase the possibility of virus transmission, mosquito lots 5 and 6 were given additional infected blood meals. Because of negative results the strain of virus used by Coggeshall<sup>16</sup> in his transmission experiments was compared with the J. P. strain. These strains were shown by means of neutralization and

transmission experiments with the former strain. In view of the fact that Huff<sup>23</sup> was unable to infect *A. aegypti* with *Plasmodium lophurae* at incubation temperatures below 28 C., and Davis<sup>25</sup> has shown that the extrinsic incubation period of yellow fever virus in *A. aegypti* was shortened at high temperatures, it was decided to determine whether *A.*  
  
25. Am. J. Hyg. 16: 163, 1932.

aegypti would successfully transmit lymphocytic choriomeningitis if incubated at constant temperatures ranging from 25 to 37 C. Results obtained in feeding experiments at various temperatures together with results of inoculating normal guinea pigs subcutaneously with ground saline suspensions of the same mosquito lots immediately after each feeding experiment are summarized in table 3.

It is evident from table 3 that mosquito incubation temperature plays an important part in the transmission of the virus by *A. aegypti* from infected to normal guinea pigs. Positive transmissions were obtained by bite with mosquitoes incubated at temperatures ranging from 26 to 34 C., but best results were obtained at 28, 30 and 32 C., while no virus was detected in mosquitoes incubated at 25 C. or lower. Successful transmission occurred from 7 to 21 days following the infective blood meal, except in lot 28 in which 5 mosquitoes transmitted a fatal infection as long as 38 days after the infective meal. As shown in table 3, results parallel to those of the feeding experiments were obtained in all but 2 instances with duplicate guinea pigs inoculated subcutaneously with ground saline suspensions of the same mosquito lots. Virus was recovered from the saline suspension of lot 18 (kept at 26 C. for 21 days), although the feeding experiment gave negative results. On the other hand, a fatal infection occurred following the bite of lot 28 (kept at 30 C. for 38 days), but no virus was detected in the saline suspension of these mosquitoes.

#### Unsuccessful Attempts to Transmit the Virus by *C. pipiens* and *A. albopictus*

Three attempts to transmit the virus from infected to normal guinea pigs by *C. pipiens* were negative. In each in-

stance small lots varying in number from 2 to 5 mosquitoes, which were kept at room temperature, fed on normal guinea pigs 5, 6, and 15 days respectively after engorging on an infected guinea pig inoculated subcutaneously 8 days previously with 0.5 cc. of a 10% virus suspension. Virus was not detected in the same lots of *C. pipiens* by subcutaneous inoculation of other normal guinea pigs with ground saline suspensions made immediately after feeding. Following an observation period of 30 days the guinea pigs were inoculated with a challenge dose of virus and succumbed in 8 to 10 days. In a fourth experiment, however, the virus was shown to survive for at least 24 hours in 3 *C. pipiens* that were ground up in salt solution about 24 hours after the infective blood meal.

Three attempts to transmit the virus from infected to normal guinea pigs by *A. albopictus* were also unsuccessful. The mosquitoes were kept at room temperature and small lots (varying in number from 3 to 6) fed on normal guinea pigs, 6, 9, and 13 days respectively after engorging on an infected guinea pig which had been inoculated subcutaneously 9 days previously with 0.5 cc. of a 10% virus suspension. Virus was not detected in the suspensions of the same lots of *A. albopictus* that were injected subcutaneously immediately after each feeding experiment into other normal guinea pigs. After a period of 30 days the guinea pigs were inoculated with a challenge dose of virus and succumbed within 8 to 10 days.

The negative transmission experiments with *C. pipiens* and *A. albopictus* incubated at room temperature (22–25 C.) are obviously inconclusive because they were completed before the importance of incubation temperature on the infectivity of *A. aegypti* was demonstrated.



Transmission of the Virus by the  
Bedbug (*C. lectularius*)

Nineteen transmission experiments with 61 guinea pigs were carried out at intervals ranging from 10 minutes to 85 days from the time of bedbug infection (table 4). Bedbugs were kept at room

Two female bedbugs (lot 8), which had engorged 17 days previously on a mouse infected 8 days before, fed on a guinea pig. Bedbug feces were deposited on the skin of the bitten guinea pig, and it died in 22 days. Pathological findings typical for lymphocytic choriomenin-

TABLE 4.—*Experimental Transmission of Lymphocytic Choriomeningitis by the Bedbug (Cimex lectularius) Kept at 22–25 C.*

| Bedbug Lot No.  | Days Between Inoculation and Feeding on Infected Guinea Pig <sup>a</sup> | Days Since Infective Blood Meal | No. of Infected Bedbugs Fed on Normal Guinea Pig |        | Bedbug Feces on Bitten Area | Results <sup>b</sup> |                 |
|-----------------|--------------------------------------------------------------------------|---------------------------------|--------------------------------------------------|--------|-----------------------------|----------------------|-----------------|
|                 |                                                                          |                                 | Male                                             | Female |                             | Days before Death    | Virus Recovered |
| 1 <sup>c</sup>  | 5                                                                        | 7                               | 0                                                | 1      | Absent                      | Lived                |                 |
| 2 <sup>c</sup>  | 7                                                                        | 14                              | 0                                                | 3      | Present                     | 28                   | +               |
| 3               | 9                                                                        | 7                               | 0                                                | 3      | Present                     | 15                   | +               |
|                 |                                                                          | 14                              | 0                                                | 3      | Present                     | 22                   | +               |
| 4 <sup>d</sup>  | 9                                                                        | 9                               | 1                                                | 2      | Absent <sup>e</sup>         | Lived                |                 |
| 5               | 8                                                                        | 23                              | 0                                                | 4      | Present                     | Lived                |                 |
| 6               | 7                                                                        | 7                               | 10 Larvae                                        |        | ?                           | 23                   | +               |
| 7               | 7                                                                        | 28                              | 0                                                | 5      | Present                     | 35                   | +               |
| 9               | 9                                                                        | 21                              | 3                                                | 0      | Present                     | 24                   | +               |
| 10              | 8                                                                        | 40                              | 1                                                | 1      | Absent                      | 14                   | 0               |
| 12              | 7                                                                        | 74                              | 1                                                | 2      | Present                     | 25                   | +               |
| 13 <sup>d</sup> | 9                                                                        | 54                              | 1                                                | 2      | Absent                      | Lived                |                 |
| 14 <sup>f</sup> | 7                                                                        | 1                               | 1                                                | 4      | Absent                      | Lived                |                 |
| 15              | 7                                                                        | 8                               | 1                                                | 1      | Present                     | 16                   | +               |
|                 |                                                                          | 85                              | 1                                                | 1      | Present                     | 21                   | +               |
| 16 <sup>f</sup> | 7                                                                        | 1                               | 1                                                | 1      | Absent                      | Lived                |                 |
| 17 <sup>f</sup> | 7                                                                        | 10 min.                         | 0                                                | 3      | Absent                      | 14                   | +               |
| 11              | Control                                                                  |                                 | 4                                                | 6      | Present                     | Lived                |                 |

<sup>a</sup> Infected guinea pigs were inoculated subcutaneously (0.5 cc.) with 10% virus suspension.  
<sup>b</sup> In negative experiments guinea pigs subsequently succumbed with typical signs of choriomeningitis following inoculation with a challenge dose of virus.  
<sup>c</sup> Bedbugs of these lots were infected since virus was recovered from a ground saline suspension of the entire lot.  
<sup>d</sup> Precautions were taken to prevent these lots from defecating on the skin of bitten guinea pigs.  
<sup>e</sup> Virus was present in feces collected from this lot.  
<sup>f</sup> Bedbugs of these lots were permitted to partially engorge for only about 2½ minutes on the infected guinea pig.

temperature (22–25 C.). One experiment demonstrating transmission of the virus from an infected mouse to a guinea pig after 16 days had elapsed is given in the following protocol. No precautions were taken to prevent bedbugs from defecating on the skin of bitten guinea pigs except lots 4 and 13.\* In order to determine if mechanical transmission of the virus may occur, lots 14, 16, and 17 were permitted to feed for only about 2½ minutes on an infected guinea pig so that engorgement would be completed on a normal animal either on the following day or after 10 minutes.

\* Defecation on the skin was prevented by elevating the bedbug container about ½ inch above the guinea pig's belly and requiring the bugs to bite through the gauze which covered the mouth of the container.

gitis were noted in brain and lung sections, and no bacteria were isolated. Recovered virus was identified by means of a neutralization test.  
The results summarized in table 4 and in the above protocol show that successful transmission of lymphocytic choriomeningitis from infected to normal guinea pigs by means of bedbugs was obtained in 11 of 18 attempts at intervals of time ranging from 10 minutes to 85 days. Both male and female bedbugs as well as first stage larvae were able to transmit the virus. Virus was detected in the ground saline suspensions of bedbug lots 1 and 2 inoculated immediately after feeding experiments, although lot 1 failed to transmit the virus by bite. It is of special interest to note that bedbug lot 3 transmitted a fatal infection

to 2 normal guinea pigs 7 and 14 days respectively after a single feeding on an infected guinea pig, and that lot 15 was able to transmit a fatal infection to 2 normal guinea pigs as long as 8 to 85

tained in 2 experiments in which the period of time between interrupted feedings was about 24 hours. These findings together with the fact that negative results were obtained in 2 experiments

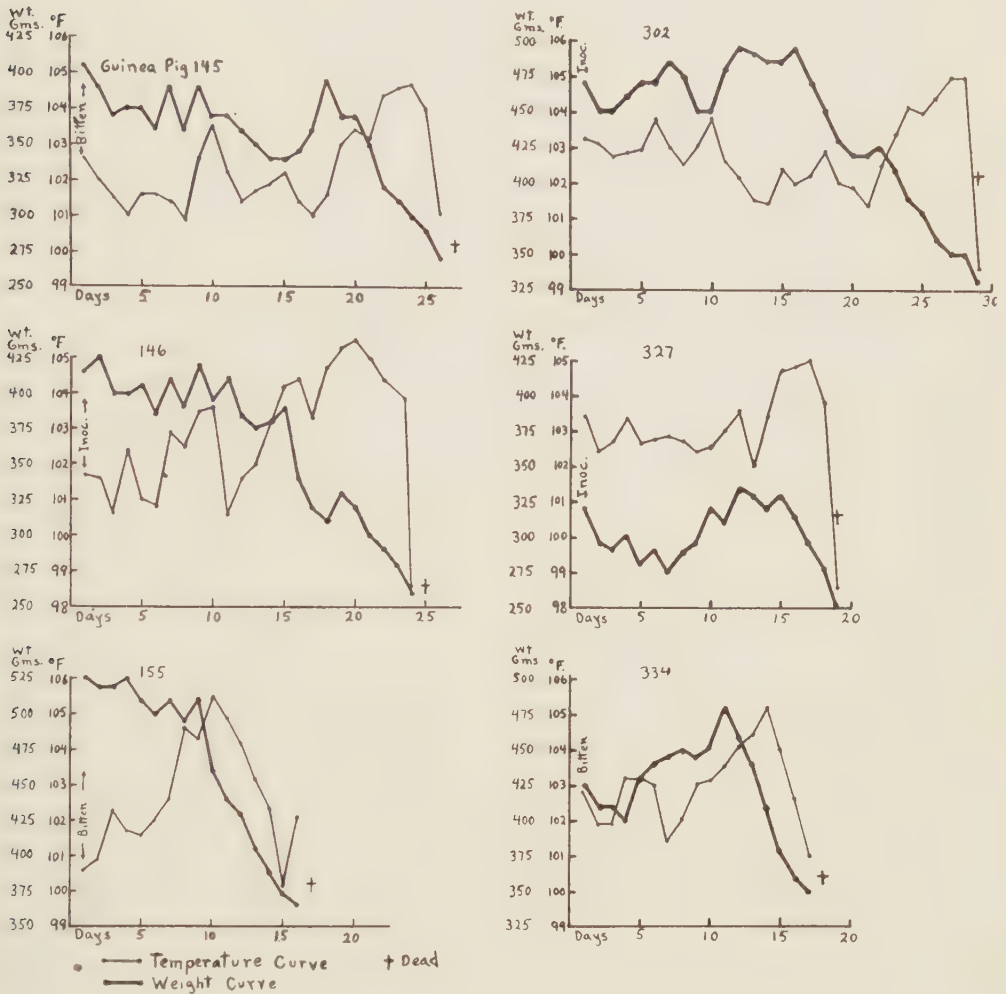


Fig. 3.—Representative typical (155, 327, 334) and atypical (145, 146, 302) daily temperature and weight curves of guinea pigs in positive arthropod transmission experiments.

days respectively after a single infective blood meal. Mechanical transmission of the virus occurred if an interval of only 10 minutes was allowed to elapse between interrupted feeding on an infected and normal guinea pig. On the other hand, negative results were ob-

in which precautions were taken to prevent bugs from defecating on the skin of bitten guinea pigs indicate that the virus does not survive on the mouth parts, and that infected bedbugs are unable to transmit lymphocytic choriomeningitis by bite alone 1, 9, and 54



days after the date of their infection. The high correlation between transmission of the virus and defecation of the bedbugs on the skins of bitten guinea pigs indicates that transmission usually occurs only when the bugs are allowed to defecate on the bitten area. Moreover, while the bedbug transmission experiments were in progress the infectivity of bug feces was frequently tested with positive results.

*Infectivity of bedbug feces.*—In four experiments it was found that virus is present in dried feces collected from infected bedbugs 3, 9, 14, and 85 days respectively from the time of infection. Feces were obtained from saline washings of test tubes in which the bugs were stored and from strips of sterile filter paper on which the bugs had defecated. In order to obtain as much of the feces as possible the strips of filter paper were soaked in physiological salt solution and rubbed in a mortar. In each instance the feces were tested for infectivity by subcutaneous inoculation into normal guinea pigs, and no bacteria were isolated from the feces suspension.

Feces collected from lots 3, 4, 5, and 15 of bedbugs which had fed 3, 9, 14, and 85 days, respectively, on infected guinea pigs, were suspended in salt solution and inoculated subcutaneously into normal guinea pigs. The animals inoculated from feces of bedbugs from lots 3 (3 days) and 15 (85 days) remained normal and were shown to be immune to challenge doses of virus sufficient to kill control animals. Guinea pigs inoculated with feces from lots 4 and 5 (9 and 14 days) died in 25 and 32 days respectively with pathological changes in brain and lung typical for lymphocytic choriomeningitis. No bacteria were isolated from either animal, and the virus recovered from each was identified by neutralization test.

*Infection of guinea pigs by rubbing*

*infected bedbug feces on the scarified skin.*—A physiological salt suspension of feces collected from bedbugs (lot 9) 8 days after feeding on an infected guinea pig was applied to the lightly scarified skin of 2 guinea pigs. At the same time a control guinea pig in a separate cage was inoculated subcutaneously with 1.5 cc. of the feces suspension. The control and the 2 test guinea pigs died in 23, 23, and 24 days respectively. Pathological findings typical for lymphocytic choriomeningitis were noted in sections of the brain and lungs, except the lungs of the control guinea pig which were apparently normal. No bacteria were isolated. Virus recovered in each instance was identified by means of neutralization tests.

*Stage to stage transmission of the virus in bedbugs.*—One experiment proved that virus may persist through molting from first to second larval stages. No virus was detected, however, when the same bedbug lot molted to the third and fourth larval stages. Ten newly hatched larvae (lot 6), which had engorged 7 days previously on an infected guinea pig, transmitted a fatal infection to a normal guinea pig (table 4). Four days after feeding on the latter, the larvae molted to the second stage and were fed 3 days later on a second guinea pig which died in 35 days. The pathological findings were typical for lymphocytic choriomeningitis in sections of the brain and lungs, and no bacteria were isolated. Recovered virus was identified by means of a neutralization test.

Eight days after infecting the second guinea pig, larva lot 6 molted to the third stage and fed on a third guinea pig. Seven days later these larvae molted to the fourth stage and fed on a fourth guinea pig. Feces were noted on the bitten area in each case. The third and fourth test guinea pigs had no pyrexia and remained normal during the

30 days that they were observed. Both animals died, 11 and 9 days respectively, following inoculation with a challenge dose of virus.

*Attempts to demonstrate hereditary transmission of the virus in the bedbug.*—Since virus was shown to persist through molting of at least one larval stage to the next, 6 experiments were performed to determine if infected females could transmit the virus to their eggs. Because of possible contamination with infected feces, the eggs were suspended in 1:1,000 mercuric chloride solution for 15 minutes and then were rinsed several times with sterile salt solution. The fact that this procedure was adequate to inactivate virus in infected feces is shown in experiment 1. Furthermore, it was found that bedbug eggs treated with 1:1,000 mercuric chloride solution for 15 minutes and then rinsed with salt solution hatched, while eggs kept in the mercuric chloride solution for 24 hours failed to do so. In experiment 2 no virus was detected in 2 batches of eggs tested soon after oviposition by infected bugs. The bedbugs were kept at room temperature during these experiments.

*Experiment 1.*—Feces collected from an infected female bedbug (lot 14), which had fed 16 days previously on an infected guinea pig, were suspended in 2 cc. of physiological salt solution. One cc. was mixed with equal parts of a 1:1,000 mercuric chloride solution in normal salt solution; after 15 minutes the mixture was inoculated subcutaneously into a guinea pig. The remaining cc. of feces suspension not treated with mercuric chloride solution was inoculated subcutaneously into a control guinea pig. The animal had no pyrexia and remained normal during the 30 days that it was observed, but it succumbed in 8 days to inoculation with a challenge dose of virus. On the other hand, the control guinea pig died in 15 days. All

findings were typical for lymphocytic choriomeningitis.

*Experiment 2.*—Ten eggs from an infected bedbug from lot 4 and 11 eggs from a bedbug from lot 14, which had previously fed 9 and 16 days respectively on an infected guinea pig, were crushed in salt solution and inoculated subcutaneously into different guinea pigs. These animals had no pyrexia and remained normal during the 30 days of observation, but died 9 and 8 days respectively after inoculation with a challenge dose of virus.

*Experiment 3.*—This was designed to test further the possibility of trans-ovarial transmission of the virus by feeding larvae recently hatched from eggs laid by an infected bug on a guinea pig and also by inoculating a ground saline suspension of them immediately after engorgement into a second guinea pig. Eleven larvae, which had hatched 4 days previously from eggs laid by an infected bedbug (lot 17), engorged on a guinea pig. Immediately after feeding the larvae were crushed in salt solution and inoculated subcutaneously into another guinea pig. Bedbug lot 17 had fed 14 days before oviposition on an infected guinea pig and hatching occurred about 14 days after the eggs were laid. The guinea pigs died in 17 and 16 days respectively. Pathological findings typical for lymphocytic choriomeningitis were noted in sections of the brains and lungs, and no bacteria were isolated. Recovered virus was identified by means of neutralization tests.

*Experiment 4.*—Thirteen larvae, which had hatched 3 days previously from eggs laid by an infected bedbug (lot 19A), and 8 larvae, 4 days after hatching from eggs laid by lot 19B, engorged on different guinea pigs. Immediately after feeding the larvae were crushed in salt solution and inoculated subcutaneously into other guinea pigs.



Lot 19A had fed 13 days and lot 19B 12 days before oviposition on an infected guinea pig. Hatching occurred about 11 days after the eggs were laid. All of the test animals remained normal during the 30 days that they were kept under observation, but died 7 or 8 days after inoculation with a challenge dose of virus.

*Transmission of the virus by bedbugs under natural conditions.*—A metal cage measuring 15.5 by 9 by 9 inches was equally divided by a solid metal partition. An infected guinea pig was put into one side of the cage and a normal guinea pig into the other. At the same time 1 male and 4 female uninfected bedbugs were liberated in the cage, and the cage was placed in a moat containing 2% cresol. A similar control cage without bedbugs was prepared with an infected guinea pig on one side of the partition and a normal guinea pig on the other. When deaths of the virus inoculated guinea pigs in both cages occurred these animals were replaced with other inoculated guinea pigs over a period of 30 days. The guinea pigs in the test cage were carefully removed for daily temperature and weight recordings so that none of the bedbugs escaped. The test guinea pig in the bedbug cage died in 31 days. The pathological, bacteriological, and immunological findings were typical for lymphocytic choriomeningitis. On the other hand, the guinea pig in the control cage without bedbugs remained normal during the 32 days that it was observed, but died 8 days following inoculation with a challenge dose of virus. These results indicate that bedbugs may transmit lymphocytic choriomeningitis from infected to normal guinea pigs under natural laboratory conditions.

*Failure to transmit the virus by feeding infected bedbugs to mice and guinea pigs.*—Twenty-two infected bedbugs (lot 18), which had fed 1 day previously on an infected guinea pig, were divided into

4 lots, and each lot was placed in a no. 5 gelatin capsule with sterile forceps. Two guinea pigs were forced to swallow gelatin capsules containing 5 and 6 bedbugs respectively. Two mice were forced to swallow the remaining 2 capsules containing 5 and 6 bedbugs respectively. The guinea pigs and mice remained normal during the ensuing 30 days. Both guinea pigs died 8 days after inoculation with a challenge dose of virus. The mice were also inoculated intracerebrally with 0.03 cc. of the same virus suspension. Both mice developed typical signs of choriomeningitis 7 days after inoculation. One mouse was found dead the next day, while the other had recovered completely 10 days after inoculation.

#### Experimental Infection of the Rhesus Monkey Louse (*E. longiceps*)

A rhesus monkey, heavily infested with lice, was inoculated subcutaneously with 2 cc. of a 10% virus suspension. Before inoculation 40 lice were removed from the monkey, ground in salt solution, and inoculated subcutaneously into a control guinea pig. No bacteria were isolated from the louse saline suspension. The control guinea pig remained normal during the 29 days that it was observed. Later it died in 9 days following inoculation with a challenge dose of virus. The monkey was killed while moribund, 14 days after virus inoculation. Four cc. of heart blood obtained from the monkey at death was inoculated subcutaneously into a guinea pig in order to determine if virus was present, with the result that this guinea pig died in 10 days. The findings were typical for lymphocytic choriomeningitis.

About 300 lice were removed from the infected monkey at the time of its death and transferred with sterile forceps to the hair on the back of an uninfected monkey to determine whether these insects transmit the virus from monkey to monkey. Before infesting the

normal monkey with the lice, a blood specimen was obtained for the detection of possible virus-neutralizing substances in the serum. The latter monkey was isolated in an individual metal cage in a room containing no infected animals. To determine if the lice were infected, 80 of them collected from the infected monkey immediately after death, were ground in salt solution and inoculated subcutaneously into 2 guinea pigs. Another guinea pig was inoculated subcutaneously with a suspension of a second lot of 80 lice also obtained from the same infected monkey immediately after death but kept in a sterile petri dish at room temperature for about 24 hours. In a preliminary experiment all lice were found dead after incubation at 37 C. for about 24 hours in an incubator having about 50% relative humidity. All guinea pigs died in 11, 11, 13 days respectively. The findings were typical for lymphocytic choriomeningitis.

None of the lice which had been placed on the monkey could be found either upon it or in its cage 5 days later. Probably the lice were eaten by the monkey. This transmission experiment was negative, since the monkey remained apparently healthy. Furthermore, choriomeningitis virus neutralizing antibodies were not detected in its blood serum at the time of louse infestation nor 32 days later. Thirty-two days after being infested with lice, this animal was inoculated with a challenge dose of virus. Seven days after inoculation monkey 153 had a fever of 104.6 F. and died 8 days later. Aerobic and anaerobic cultures of the heart blood were bacterially sterile.

#### Experimental Infection of the Blood-Sucking Mite (*A. glasgowi*)

Four attempts to transmit the virus by uncontrolled feeding of infected mites on mice at intervals ranging from a few

minutes to 25 days gave negative results. The virus, however, was shown to survive for at least 25 days in mites kept at room temperature in experiment 2 of this series. In every instance no bacteria were isolated from ground up mites suspended in salt solution.

*Experiment 1.*—A mouse inoculated 4 days previously with 0.5 cc. of a 10% virus suspension was put into a glass jar containing about 200 uninfected adult and larval mites. Almost immediately the mites crawled on the inoculated mouse and disappeared among its hairs. Two days later the mouse developed typical signs of choriomeningitis and recovered 4 days later. Seventeen mites removed from the hair of the mouse 10 days after virus inoculation were ground in salt solution and inoculated subcutaneously into a guinea pig, which died 11 days later. The findings were typical for lymphocytic choriomeningitis.

Two additional lots of mites numbering approximately 60 each were also removed from the mouse 10 days after virus inoculation. One lot was transferred immediately to the hair on the back of a mouse; the second lot was kept in a test tube at 22–25 C. for a period of 8 days and then transferred to the hair on the back of a second mouse. The mice were isolated in individual jars. The first mouse remained normal during the 30 days that it was kept under observation, but died 9 days following intracerebral inoculation (0.03 cc.) of a challenge dose of virus. The second mouse developed a rough coat and anorexia 3 days after exposure to the mites and died 2 days later. No bacteria were isolated from the brain tissues or heart blood. A 10% suspension of the brain tissues in buffered salt solution was prepared and injected into a guinea pig. This animal remained normal during the ensuing 30 days, but died 10 days after inoculation with a challenge dose of virus.



*Experiment 2.*—A mouse inoculated 5 days previously with 0.5 cc. of a 10% virus suspension was introduced into a glass jar containing about 300 uninfected adult and larval mites. Two days after exposure to the mites the mouse developed typical signs of choriomeningitis and died 4 days later. Twenty mites removed from hairs on the body of the mouse soon after death were stored in a test tube at 22–25 C. for 25 days and then were ground in salt solution and inoculated subcutaneously into a guinea pig, which died 17 days later. The findings were typical for lymphocytic choriomeningitis.

Two additional lots of mites numbering approximately 80 each were also removed from the dead mouse and stored for 25 days. The 2 lots were then transferred to the hair of 2 mice which were isolated in individual glass jars. Both mice appeared normal during the 30 days that they were observed, but died 8 and 10 days after intracerebral inoculation (0.03 cc.) of a challenge dose of virus.

*Transmission of the virus by feeding infected mites to mice and guinea pigs.*—A mouse inoculated 5 days previously with 0.5 cc. of a 10% virus suspension was introduced into a glass jar containing about 100 uninfected adult and larval mites. One day later the mouse developed typical signs of lymphocytic choriomeningitis and recovered 5 days later. In order to determine if the mites were infected, 20 were collected from hair of the mouse 11 days after virus inoculation, ground in salt solution, and inoculated subcutaneously into a normal guinea pig which died 17 days later. The findings were typical for lymphocytic choriomeningitis.

Eighty mites also removed from the mouse 11 days after virus inoculation were divided into 4 lots, and each lot was placed in a gelatin capsule. Two

mice and 2 guinea pigs were each forced to swallow a gelatin capsule containing 20 living, infected mites. Both mice developed typical signs of choriomeningitis 7 days after swallowing the infected mites but recovered about 6 days later. Thirty days after the feeding experiment the mice were immune to intracerebral inoculation with a challenge dose of virus, while 2 control mice inoculated simultaneously died in 7 days. The guinea pigs died in 20 to 25 days respectively. The findings were typical for lymphocytic choriomeningitis.

#### DISCUSSION

In confirming Coggeshall's work on the transmission of lymphocytic choriomeningitis by *A. aegypti* it was found that transmission could not be demonstrated unless the mosquitoes were incubated at temperatures ranging from 26 to 34 C. These results are not directly comparable with those of Coggeshall because he failed to indicate incubation temperatures in his transmission experiments. Furthermore, Coggeshall obtained transmission by *A. aegypti* 15 days after the date of their infective meal. In the present studies successful transmission by bite was obtained as long as 38 days after the date of infection.

Four preliminary attempts to transmit the choriomeningitis virus by *A. aegypti* kept at room temperature (22–25 C.) gave uniformly negative results. In addition to the work by Huff already mentioned Davis<sup>25</sup> has shown that the extrinsic incubation period of yellow fever virus in *A. aegypti* was shortened at high temperatures and prolonged at low temperatures. The time required for mosquitoes incubated at various constant temperatures to become infective was as follows: 4 days at 37 C.; 8 days at 25.1 C.; 11 days at 23.4 C.; 18 days at 21 C.; and more than 30 days

at 18 C. Davis also proved that there was no attenuation of yellow fever virus in the insect host after storage for 4 weeks at 8 C. because the same mosquitoes became highly infective when they were subsequently reincubated at 36 C. for 6 days. There was, however, evidence that virus may have been attenuated in mosquitoes after exposure to relatively high temperatures (20 days at 36 C. and 7 hours at 40 C.). In the present studies no virus was detected in ground saline suspensions of *A. aegypti* incubated at 25 C. and lower, or at 37 C.

There is much evidence emphasizing the importance of temperature in the transmission of disease by arthropod vectors. The effect of temperature on the development of the sexual cycle of the 3 species of human malaria parasites within their anopheline vectors is well known. Blanc and Caminopetros<sup>26</sup> found that exposure to 16.4 C. of *A. aegypti* which have bitten a dengue fever patient renders them non-infective. If the temperature is raised to 22 C., the power to infect is restored. More recently Inada<sup>27</sup> reported that the virulence of Japanese B encephalitis from infected mosquitoes (*Culex tritaeniorhynchus* and *C. pipiens* var. *pallens*) is affected by the temperature at which the mosquitoes are kept. Better results in transmission were obtained with mosquitoes that had been kept at temperatures of 27 to 30 C. or at 27 to 32 C. than with those kept at 20 to 25 C. or at 16 to 26 C. According to Aragão<sup>28</sup> the "vital optimum" of *A. aegypti* lies between 27 and 32 C. This author states that the physiological activities of this species are retarded at

temperatures between 25 and 17 C. and cease at lower temperatures. Therefore, it is reasonable to assume that development of infectivity is retarded or stopped in the insect host at lower temperatures because metabolic activities are unfavorable for the survival or multiplication of the virus. The results obtained in the present studies justify repetition of transmission experiments with certain virus diseases suspected of being insect-borne, such as poliomyelitis and St. Louis encephalitis with insect incubation temperatures carefully controlled.

Under the conditions of the experiments reported in this study widely separated arthropod species—*A. aegypti*, bedbugs (*C. lectularius*), rhesus monkey lice (*E. longiceps*), and a species of blood-sucking mite (*A. glasgowi*)—were successfully infected with the virus of lymphocytic choriomeningitis. It was unnecessary to employ temperatures above 25 C. to infect these arthropods with the virus except in *A. aegypti* experiments. Because of the relatively high temperatures necessary for infection, *A. aegypti* would probably not be a vector of any importance in nature. Furthermore, these results suggest that survival of the virus in these arthropods is probably more dependent on adaptation of the physiological processes of the arthropod to a given temperature range than on environmental temperature per se. In this connection Aragão<sup>28</sup> points out that the yellow fever virus multiplies in jungle mosquitoes, although the optimum temperature range of the latter is relatively much lower than that of *A. aegypti*.

The experiments on infectivity of bedbug feces\* are significant because bed-

26. Ann. Inst. Pasteur **44**: 367, 1930.

27. Bull. Office internat. d'hyg. pub. **29**: 1389, 1937 (cited in Matheson Commission: Epidemic Encephalitis, New York, Columbia University Press, 1939, p. 176).

28. Mem. Inst. Oswaldo Cruz **34**: 575, 1939.

\* Since these experiments were completed, Armstrong (Harvey Lectures, Lancaster, Pa., Science Press Printing Co., 1941, p. 39) has reported negative results in attempts to transfer



bugs habitually defecate while feeding.<sup>29</sup> It appears possible that bedbugs might be a vector in the transmission of lymphocytic choriomeningitis in nature or in animal quarters infested with these insects. As mentioned before, white and gray mice, rhesus monkeys, dogs, and man have been found naturally infected with lymphocytic choriomeningitis, but the manner in which infection was acquired is unknown. Bedbugs are known to feed readily on these and other animals as well as man.<sup>29</sup>

It is interesting to note that bedbugs (*C. lectularius* and *Cimex hemipterus*) have not been definitely incriminated as important natural vectors of any disease.<sup>30</sup> Experimentally, various species of *Leishmania* can develop in bedbugs. Bedbugs are also capable of transmitting bubonic plague, relapsing fever, tularemia, and Chagas' disease. Manteiro<sup>31</sup> was able to detect yellow fever virus in the feces of bedbugs 2 to 12 days after allowing them to bite infected rhesus monkeys. In one of 16 tests Howard and Clark<sup>32</sup> recovered the poliomyelitis virus in the filtrates of bedbugs which had fed 7 days previously on an infected monkey.

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infection by bedbugs, rat and mice fleas, and a bloodsucking mite (species not stated). Furthermore, he was unable to detect virus in each species when the engorged arthropods were emulsified and inoculated into mice at intervals longer than 1 hour after the infective feeding. We are unable to account for discrepancy in results because Armstrong gave no details of his experiments. There are many factors which might explain failure to transmit the infection, such as virus strain and virulence, arthropod incubation temperature, and arthropod species and strain.

29. Matheson, R.: *Medical Entomology*, Springfield, Charles C Thomas, 1932, p. 127.

30. Hegner, R., Root, F. M., Augustine, D. L., and Huff, C. G: *Parasitology*, New York, D. Appleton-Century Co., 1938, p. 654.

31. *Brasil-med.* **43**: 1037, 1929 (cited by Matheson).<sup>29</sup>

32. *J. Exper. Med.* **16**: 805, 1912.

The rhesus monkey louse was considered as a possible vector of the virus for the following reasons: (1) stock rhesus monkeys have been found naturally infected with the virus; (2) Coggeshall<sup>16</sup> reported a lymphocytic choriomeningitis epizootic in monkeys used for the study of experimental malaria that resulted in the death of 11 animals; and (3) I was accidentally infected while attempting to transmit the virus from monkey to monkey by *E. longiceps*.<sup>20</sup> About 5 days before onset of illness, I found several lice on my hand from a monkey dying of lymphocytic choriomeningitis. Other lice which were recovered from the infected monkey on the same day were ground in salt solution and inoculated subcutaneously into a guinea pig. This animal developed typical signs of lymphocytic choriomeningitis and died in 10 days. Recently Armstrong<sup>33</sup> has announced successful transmission of infection in 2 of several trials by transferring approximately 100 lice taken from an infected monkey to a normal one.

The successful transmission of lymphocytic choriomeningitis to mice and guinea pigs swallowing infected mites is of significance because few workers<sup>4,6,8</sup> have been able to infect mice and guinea pigs by feeding virus-infected tissues. Perhaps virus-infected tissues fed to susceptible animals is destroyed by digestive processes, while within infected mites possibly it is protected from such activities so that infection may occur. Shaughnessy and Zichis<sup>6</sup> have shown that infection is produced when virus is applied to the mucous membranes of the rectum. Why transmission did not occur when mice and guinea pigs swallowed living, infected bedbugs cannot be explained other than that protection from digestive processes afforded by these in-

33. *Harvey Lectures*, Lancaster, Pa., Science Press, 1941, p. 39.

sects may have been inadequate. Findlay and MacCallum<sup>34</sup> found that acid gastric juice inactivates the yellow fever virus, and they were able to infect rhesus monkeys and the African guenon (*Cercopithecus aethiops*) with infected material administered by means of a catheter passed into the stomach.

While it is not known whether or not the virus multiplies in the various arthropods successfully infected, the results obtained may be explained largely on the basis of mechanical transmission without multiplication. First, in the neutralization tests to identify recovered virus it was noted that guinea pigs inoculated subcutaneously with high dilutions of virus ( $10^{-4}$  and  $10^{-5}$  dilutions of infected guinea pig brain suspension) sometimes had prolonged and variable incubation periods and length of life in contrast to guinea pigs inoculated with lower dilutions of virus. Similarly, there was a prolonged and variable incubation period (7 to 25 days) and length of life (16 to 30 days) of guinea pigs in positive arthropod transmission experiments. Typical and atypical daily temperature and weight curves are shown in figure 3. Second, the virus has been shown to remain active for at least 6 days at 22 C.<sup>13</sup> It was shown that virus may persist through molting from first to second larval stages but not when the same bedbug lot molted to the third and fourth larval stages. On the other hand, bedbug lot 3 transmitted a fatal infection to 2 guinea pigs after a single feeding on an infected guinea pig, and bedbug lot 15 was able to transmit a fatal infection as long as 8 and 85 days respectively after a single infective blood meal. Perhaps these results might be explained in part at least by the highly virulent strain of virus employed in the present studies.

Ground saline suspensions of bedbugs,

monkey lice, and mites as well as bedbug feces were consistently bacterially sterile, presumably due to the presence of a bactericidal principle in the alimentary tract of these arthropods as described by Duncan.<sup>35</sup> On the other hand, saprophytic bacteria such as *Bacterium aerogenes* and nonpathogenic diphtheroids were isolated from ground mosquito suspensions. In general these results agree with those reported by Duncan.<sup>35</sup>

#### SUMMARY AND CONCLUSIONS

In a guinea pig subcutaneously inoculated with a virus-infected brain suspension of lymphocytic choriomeningitis the virus could be recovered from the blood 24 hours later, its concentration gradually increased to a maximum on the fifth day, and persisted until the death of the animal. Likewise the virus was detected in the blood of mice after 24 hours and persisted for at least 7 days following either intracerebral, subcutaneous, or intraperitoneal inoculations.

The transmission of lymphocytic choriomeningitis by *A. aegypti* occurred by bite when the mosquitoes were incubated at various constant temperatures ranging from 26 to 34 C., while no virus was detected in mosquitoes incubated at 25 C. and lower, or at 37 C. Best results in transmission were obtained with mosquitoes kept at 28, 30, and 32 C. Successful transmission occurred at periods of time varying from 7 to 38 days following the infective blood meal.

Six attempts to transmit lymphocytic choriomeningitis from infected to normal guinea pigs by *C. pipiens* and *A. albopictus* incubated at room temperature (22–25 C.) at intervals ranging from 5 to 15 days were unsuccessful.

Successful transmission of lymphocytic choriomeningitis from infected to

34. J. Path. & Bact. **49**: 53, 1939.

35. Parasitology **18**: 238, 1926.



normal guinea pigs by means of bedbugs (*C. lectularius*) incubated at 22–25 C. was obtained in 11 of 18 attempts at intervals of time ranging from 10 minutes to 85 days. In one instance transmission of the virus from an infected mouse to a normal guinea pig by bedbugs after 16 days had elapsed was demonstrated. Both male and female bedbugs as well as first stage larvae were able to transmit the virus. Evidence was presented showing that bedbugs are unable to transmit infection by bite alone and that transmission usually occurs only when the bugs are allowed to defecate on the bitten area. Virus was detected in dried feces collected from infected bedbugs as long as 85 days from the time of infection. Infection was also transmitted by rubbing infected bug feces on the lightly scarified skin of normal guinea pigs. One experiment was done showing that virus may persist through molting from first to second larval stages but not when the same bedbug lot molted to the third and fourth larval stages. Transovarial transmission of the virus to subsequent larvae was demonstrated in one

instance but 2 attempts to repeat this experiment were unsuccessful. Attempts to transmit infection by forcing mice and guinea pigs to swallow living, infected bedbugs in gelatin capsules failed. One successful transmission experiment was done under conditions which approximated those occurring in nature.

One attempt to transmit lymphocytic choriomeningitis from an infected to a normal rhesus monkey by uncontrolled feeding of rhesus monkey lice (*E. longiceps*) failed. The virus, however, was shown to survive for at least 24 hours in infected monkey lice incubated at 22–25 C.

Four attempts to transmit lymphocytic choriomeningitis by uncontrolled feeding of infected mites (*A. glasgowi*) incubated at room temperature on normal mice at intervals ranging from a few minutes to 25 days gave negative results. The virus, however, was shown to survive for at least 25 days in infected mites. Successful transmission was obtained by forcing mice and guinea pigs to swallow living, infected mites in gelatin capsules.











